Memoir No. 25. *August, 1932.*

INDIAN MEDICAL RESEARCH MEMOIRS

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TO THE

INDIAN JOURNAL OF MEDICAL RESEARCH

REPORTS OF THE KALA-AZAR COMMISSION, INDIA

REPORT No. II (1926–1930).

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TRANSMISSION EXPERIMENTS IN INDIAN KALA-AZAR WITH *PHLEBOTOMUS ARGENTIPES.**

As soon as the work of Knowles, Napier, and Smith (1924) had shown that the parasite of Indian kala-azar flagellated in the midgut of *Phlebotomus argentipes* it became of the first importance to determine the part played, if any, by this insect in the transmission of the disease. The mere occurrence of flagellation of *Leishmania donovani* in the midgut of the fly gave no indication as to the direction in which research should be directed in order to determine the mechanism of transmission.

Transmission might have occurred, on the analogy of some other protozoal diseases, by the bite of the sandfly, but, at the time no knowledge was available as to any process by which flagellates present in the midgut of the insect could be transferred to the wound caused by its bite. Transmission, on the other hand, might have occurred by the squashing of the fly on the skin at the time of its second feed, leading to the contamination of the wound by the flagellates present in the midgut as the result of the first feed. Other means of transmission might have been the ejection, at the time of feeding, of fæces containing flagellates on to the wound made in biting, or the contamination of the hands, food, or water by possible encysted forms of the flagellate voided with its dejecta.

The gap in our knowledge which was the cause of this uncertainty was bridged by the working out by the Kala-azar Commission of the complete life-history of *Leishmania donovani* in *Phlebotomus argentipes*, a knowledge of which immediately focussed attention on what was the most probable mode of transmission from insect to vertebrate. It was pointed out in this description of the life-history of the flagellate that, from the time of the ingestion by the fly of Leishman-Donovan bodies from the peripheral blood of a case of kala-azar, and the development of these bodies into flagellates, there was a tendency to the progressive development, in an anterior direction, of the flagellate infection, until this had in some cases extended up to the mouth proper of the insect. These facts led to the presumption that infection must be conveyed by the bite of the insect.

Technique of the experiments.—Cases of kala-azar showing the largest number of parasites in the peripheral blood were selected to be fed upon by the flies, the blood being examined as described below.

A small drop of blood is placed at one end of a slide ; a second slide is applied to it, as in making an ordinary blood smear. The second slide, as soon as the blood has spread out along its edge, is pushed along the surface of the first with an even motion until the blood is almost exhausted. At this point, instead of continuing this motion, as in making an ordinary smear, the second slide is abruptly lifted off, with the result that the blood smear ends in a straight edge stretching transversely across the slide. This straight edge is somewhat thicker than the rest of the smear and contains a large percentage of the total white cell content of the

* Shortt, H. E., Barraud, P. J., and Craighead, A. C. *Ind. Jour. Med. Res.*, **14**, No. 3, 1927.

drop of blood. The white cells in the straight edge are all that it is necessary to examine for the purpose of determining, with a fair degree of accuracy, the presence and numbers of Leishman-Donovan bodies in the peripheral blood.

The sandflies were fed on the selected cases of kala-azar in the manner described by us in another communication and were subsequently kept continuously at a temperature of 28°C. After the lapse of three or four days the flies had oviposited, and were ready for their second feed. This was given them either on the same or another kala-azar case, or on an experimental animal such as the mouse or rabbit, the latter being the more convenient. The third and subsequent feeds were always on experimental animals, which were either white mice or the Chinese hamster *Cricetulus griseus.**

After the last feed of the fly the latter was examined, if in a suitable condition, either by dissection or by the cutting of microscopical sections, to determine whether it was an infected specimen. The last stage of the experiment consisted in the examination of the experimental animals by direct microscopic and cultural methods for the presence of infections.

The details of the experiments are summarized in Tables I, II and III :—

Summary of Table I.

Showing the results of experiments in which white mice were subjected to the second feed of P. argentipes.

Number of animals (white mice) used.	Total number of feeds.	Number of flies known to be infected.	Number of flies known to be not infected.	Number of flies—condition unknown (not dissected).	Result.
94	129	12	46	71	All negative.

Summary of Table II.

Showing the results of experiments in which white mice were subjected to the third and subsequent feeds of P. argentipes.

Number of animals (white mice) used.	Total number of feeds.	Number of flies known to be infected.	Number of flies known to be not infected.	Number of flies—condition unknown (not dissected).	Result.
89	90	33	56	1	All negative.

* We are indebted for the opportunity of employing hamsters in our experiments to the kindness of Dr. C. W. Young, M.D., of the Peking Union Medical College, who, at great personal trouble and inconvenience, imported a supply of these animals from China for our use.

SUMMARY OF TABLE III.

Showing the results of experiments in which Chinese hamsters Cricetulus griseus *were subjected to the third and subsequent feeds of* P. argentipes.

Number of animals (Chinese hamsters) used.	Total number of feeds.	Number of flies known to be infected.	Number of flies known to be not infected.	Number of flies—condition unknown (not dissected).	RESULT.
20	603	140	432	31	All negative.

Summary of details given in Tables I, II and III.—A study of the Tables will reveal the fact that, in all, 203 experimental animals have been fed upon 822 times by 691 flies which had previously fed upon kala-azar cases showing parasites in their peripheral blood. Of these flies 152 were proved by subsequent examination to be infected individuals, and 435 were found uninfected. In the case of 104 their condition was not known, but this number must have contained many infected flies. The actual number of feeds by flies known to be infected was 184, which is considerably greater than the number of infected flies, as some of the flies were fed more than once on the experimental animals. In the case of Table III, giving the details of feeding on hamsters, it is seen that the animals, with 3 exceptions, had a minimum of 3, and a maximum of 15 flies, known to be infected, fed on individuals. Our experience in the dissection of flies caught in nature in houses containing kala-azar cases makes it extremely improbable that any such intensive feeding of infected flies on man should occur under natural conditions. The hamsters, therefore, may be said to have been submitted to at least the maximum degree of exposure to infection by the bite of the sandfly that is ever likely to occur in nature in the case of man over the same limited period of time.

Conclusions.—Sixty experimental animals, subjected to 184 bites from a minimum of 152 *Phlebotomus argentipes* known to be infected with *Leishmania donovani*, have in no case developed kala-azar.

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